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Biosynthesis of the third component of complement (C3) by the human monocytic-cell line U-937

Induction by phorbol myristate acetate

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Phorbol myristate acetate (PMA)-stimulated human monocyte-like cells (U-937) were found to synthesize the third component of complement (C3), as shown by enzyme-linked immunosorbent assay and immunoprecipitation from [^{35}S]methionine-labelled culture supernatants. C3 synthesis occurred at a rate of about 160 ng of C3/24 h per 10^6 cells on day 7 after addition of PMA; it was blocked by cycloheximide treatment and was restored after removal of the inhibitor. SDS/polyacrylamide-gel-electrophoretic analysis of the immunoprecipitated protein showed that the size and subunit structure of the newly synthesized C3 were identical with those of plasma C3, and that a single-chain intracellular precursor was present in the cell lysates. Haemolytic assays showed that the synthesized C3 fully expressed functional activity in early culture within 4 h. After longer culture, a loss of haemolytic activity was observed. The possibility that newly secreted C3 is cleaved by U-937 cells themselves was suggested.

INTRODUCTION

U-937 is a human histiocytic cell line that possesses many monocyte characteristics, such as surface antigens (Mosciki *et al.*, 1983), receptors, including Fc and complement receptors (Sundstrom & Nilsson, 1976), production of lysozyme, esterases (Sundstrom & Nilsson, 1976) and elastase (Senior *et al.*, 1982) and synthesis of interleukin 1 (Palacios *et al.*, 1982). This cell line can be differentiated into macrophages by various agents, in particular phorbol myristate acetate (PMA), which is capable of inducing functional and maturational changes in various macrophage cell lines (Koren *et al.*, 1979; Rovera *et al.*, 1979; Hattori *et al.*, 1983; Feuerstein & Cooper, 1984). The cells of the monocyte/macrophage lineage are known to produce complement components. Monocytes and macrophages can synthesize C1 (Muller *et al.*, 1978; Bensa *et al.*, 1985), C2 (Einstein *et al.*, 1976; Beatty *et al.*, 1981), C3 (Whaley, 1980; Strunk *et al.*, 1983; Koestler *et al.*, 1984), C4 (Lai *et al.*, 1975; Colten, 1982), C5 (Hartung & Hadding, 1983) and all the components of the alternative pathway (Whaley, 1980; Hartung & Hadding, 1983; Ezekowitz *et al.*, 1983). C2 and C4 biosynthesis has been extensively studied, but fewer data are available for C3, especially concerning its structural form and kinetics of synthesis. The synthesis of complement components by U-937 cells has been investigated (Minta & Pambrun, 1983), but only C2 (Littman *et al.*, 1983) and factor D production (Barnum & Volanakis, 1985) has been well documented.

We investigated the synthesis of the third component of complement (C3) by U-937 cells after their differentiation into macrophage-like cells by PMA.

MATERIALS AND METHODS

Materials

RPMI 1640, foetal-calf serum (FCS), penicillin and streptomycin were purchased from Boehringer Mannheim, Mannheim, Germany. PMA, cycloheximide and *p*-nitrophenyl phosphate disodium salt were obtained from Sigma, Paris, France; [^{35}S]methionine (specific radioactivity 800 Ci/mmol) and ^{14}C-labelled proteins were from Amersham International, Les Ulis, France; protein A–Sepharose was from Pharmacia, Bois d'Arcy, France; and Aqualuma was obtained from Kontron, Velizy, France.

Cell culture

The U-937 cell line (a gift from Dr. J. C. Bensa, INSERM U-238, Grenoble, France) was grown in suspension in RPMI 1640 supplemented with 10% (v/v) FCS, penicillin (100 units/ml) and streptomycin (100 μg/ml). Culture took place in 75 cm^2 flasks in a CO_2/air (1:19) atmosphere at 37 °C. The cell density was 5×10^5 cells/ml.

Differentiation into 'macrophage-like' cells was induced by incubating 5×10^6 cells in 10 ml of medium containing 0.1 μg of PMA/ml. The flasks were then left at 37 °C in the presence of PMA for at least 3 days without harvesting.

Portions or whole medium were then harvested every 24 h and kept at −80 °C before study. In some experiments, portions of medium were harvested every hour for investigation of the kinetics of C3 synthesis over a 6 h period.

Abbreviations used: PMA, 4-phorbol 12-myristate 13-acetate; e.l.i.s.a. enzyme-linked immunosorbent assay; PMSF, phenylmethanesulphonyl fluoride; DTT, dithiothreitol; DMSO, dimethyl sulphoxide; PPO, 2,5-diphenyloxazole; FCS, foetal-calf serum; Con A, concanavalin A.

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Inhibition of C3 synthesis by cycloheximide was studied in some experiments. Cycloheximide was dissolved in RPMI 1640, filtered through a 0.22 μm-pore-size filter (Millipore) and added to the cell cultures at a concentration of 5 μg/ml. To assess the recovery of synthesis, U-937 cells were incubated for 6 h with the inhibitor, washed twice in RPMI [previously equilibrated at 37 °C, CO_2/air (1:19), for 2 h in the incubator] and resuspended in RPMI/10% (v/v) FCS medium without cycloheximide.

E.l.i.s.a. assay for C3

Highly purified C3 was obtained as described by Al Salihi *et al.* (1982) and was coupled to Sepharose 4B by the CNBr method of March *et al.* (1974). Anti-C3c was obtained by injection of purified C3c into rabbits, and monospecific antibodies were recovered after affinity chromatography on a Sepharose–C3 column in conditions outlined in Fontaine *et al.* (1980). Rabbit anti-(human C3c) was alkaline phosphatase-conjugated as described by Avrameas (1969).

E.l.i.s.a. was performed in 96-well, flat-bottomed, plates coated with 250 μl of rabbit anti-(human C3c) IgG (10 μg/ml in 0.1 M-$NaHCO_3$ buffer, pH 9)/well overnight at 37 °C. The plates were then washed three times by immersion in phosphate-buffered saline [0.15 M-NaCl/0.01 M-Na_2HPO_4/0.01 M-NaH_2PO_4(pH 6.8)]/0.01% (v/v) Tween 20, and 250 μl of phosphate-buffered saline/0.5% (v/v) bovine serum albumin were added to each well and left for 3 h. After three washings in phosphate-buffered saline/Tween, the culture supernatants (250 μl/well) were added and left overnight. After three washings in phosphate-buffered saline/Tween, the alkaline phosphatase-conjugated anti-C3 was added [250 μl/well diluted 33 μg/ml in phosphate-buffered saline/5% (v/v) bovine serum albumin] and left for 4 h at 37 °C. The plates were then washed three times in phosphate-buffered saline/Tween and 200 μl of phosphatase substrate solution were added to each well [*p*-nitrophenyl phosphate disodium salt (1 mg/ml) in a 1 mM-$MgCl_2$/1 M-diethanolamine buffer, pH 9.8). After 30 min at room temperature, the A_{405} was measured in each well with an e.l.i.s.a. reader.

Human purified C3 was used as standard in the e.l.i.s.a.; the A_{405} was proportional to the C3 content of a sample over the range 3–100 ng/ml. A C3-depleted serum (Fontaine *et al.*, 1980) was checked in a control assay and showed no significant absorbance.

Biosynthetic labelling

'Macrophage-like' U-937 cells (differentiated by at least 4 days of culture in the presence of PMA) were used for biosynthetic labelling. The cells were left for 1 h in methionine-free RPMI 1640 medium (without FCS), and 100 μCi of [^{35}S]methionine were added/ml; different incorporation times were studied. At the end of the incorporation periods, portions of supernatants were harvested and immunoprecipitation was performed immediately. For pulse–chase experiments, the labelled medium was removed after 1 h of incorporation of [^{35}S]methionine (100 μCi/ml), and the cells were left in RPMI 1640 supplemented with 3 mM unlabelled methionine for various periods.

At the end of the biosynthetic labelling, the cells were washed twice in RPMI 1640 and lysed by addition of 2 ml of cold lysis buffer [0.05 M-Tris/HCl/1 mM-PMSF/0.4% (w/v) Triton X-100, pH 7.5]. The lysates were collected, centrifuged 15 min at 10000 *g* and immediately analysed by immunoprecipitation.

Immunoprecipitation

The radiolabelled supernatants and cellular lysates were immunoprecipitated with affinity-purified anti-(human C3c) (1 mg/ml) coupled to protein A–Sepharose. A 4 ml portion of supernatant was mixed with 50 μl of protein A–Sepharose (diluted 1:2 in phosphate-buffered saline) and 20 μl of rabbit anti-(human C3c) IgG, and the mixture was incubated overnight at 4 °C with shaking. Ten washings were then performed: five in buffer A [0.05 M-Tris/HCl/0.15 M-NaCl/0.1% (w/v) Triton X-100/0.1% (w/v) deoxycholate/1 mM-PMSF, pH 7.2] and five in buffer A+1 M-NaCl. The immunoprecipitates were dissolved in 50 μl of 0.25 M-Tris/HCl/2% (v/v) SDS/5% (v/v) glycerol/0.4 M-DTT, and boiled for 5 min. A 10 μl portion of the eluate was mixed with 2 ml of Aqualuma and counted for radioactivity in an LKB counter as a control, and the remaining sample was analysed by SDS/polyacrylamide-gel electrophoresis.

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970), a resolving gel of 10% (w/v) acrylamide and a stacking gel of 3% acrylamide being used. Electrophoresis was carried out at 10 mA/gel overnight at room temperature in the LKB vertical electrophoresis unit. The following ^{14}C-labelled proteins were run in the gels for estimation of M_r: myosin (M_r 200000), phosphorylase *b* (94000), bovine serum albumin (69000), ovalbumin (43000), carbonic anhydrase (30000) and lysozyme (14300).

For autoradiography, the gells were impregnated with PPO in DMSO as described by Bonner & Laskey (1979), then dried and exposed for 3 days–1 week to Fuji X-Ray film.

C3 haemolytic assays

C3 haemolytic assay was performed as fully described by Fontaine *et al.* (1980). Briefly, 0.5 ml of sheep erythrocytes sensitized with rabbit anti-(sheep erythrocyte) antibodies at 10^8 cells/ml was mixed with 0.5 ml of an R3 reagent (C3-depleted serum, prepared as described above) diluted 20-fold and 0.5 ml of the source of C3 for 1 h at 37 °C. The reaction was stopped by addition of 3.5 ml of cold phosphate-buffered saline. A range of dilutions of fresh normal serum was assessed in parallel as a control; the erythrocyte lysis was estimated by measurement of the A_{412} after centrifugation, and the percentage of lysis (y) was estimated for each sample. The number of haemolytic sites, Z, was calculated according to the equation:

$$Z = -\ln(1-y)$$

as well as the number of effective molecules.

RESULTS

Biosynthesis of C3 by stimulated U-937 cells

U-937 cells were either grown to exponential phase or treated with PMA. The culture medium was changed every 24 h. When exposed to PMA, gross morphological changes consisting of clumping of cells and adherence to

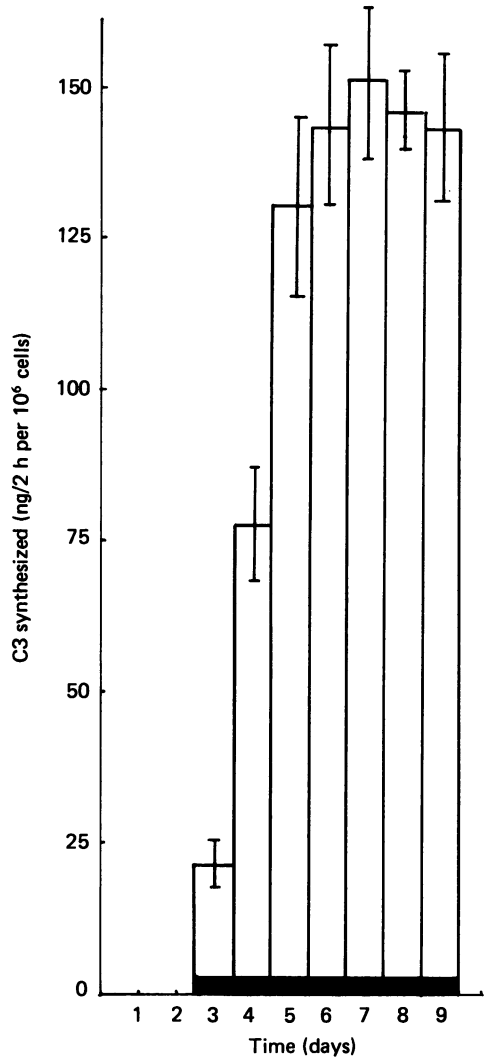

Fig. 1. Effect of PMA on C3 synthesis by U-937 cells

The cells (5×10^5/ml) were grown in the presence (▭) or in the absence (■) of PMA, and the medium was changed every 24 h. This Figure represents the production of C3 in the culture medium as assessed by e.l.i.s.a. (results are means ± S.E.M. for six experiments).

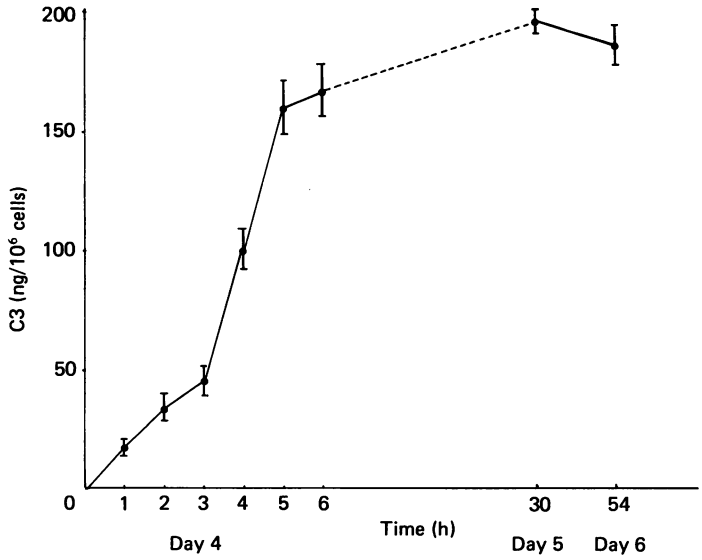

Fig. 2. 'Short-time' kinetics of C3 synthesis

The U-937 cells were grown for 4 days in the presence of PMA. On day 4, the whole medium was changed and portions of supernatant were harvested every hour for 6 h. The medium was not changed, and portions of supernatant were harvested at 30 h (day 5) and 54 h (day 6). All samples were assayed by e.l.i.s.a. in triplicate (results are means ± S.E.M. for three experiments).

plastic surfaces were observed within 1 day of culture and was virtually complete at the end of 3 days. This treatment resulted in slower growth of cells (about 76% inhibition) (Hattori *et al.*, 1983). Only few cells were not adherent and were removed during first medium changes. U-937 cells always retained more than 90% viability, as shown by Trypan Blue exclusion. C3 was measured in supernatants of U-937 cultures up to 10 days. The culture medium was completely changed every 24 h. As shown in Fig. 1, whereas no detectable amount of C3 (within the limit of our assay) was produced by untreated U-937 cells, significant and consistent synthesis of C3 was detected after 3 days in supernatants of PMA-treated cells. C3 production increased until day 7 and reached a plateau value of 162 ± 14 ng of C3/24 h per 10^6 cells. After day 9, a decrease in C3 production was observed, and a large proportion of the cells lost their adherence and died.

'Short time' kinetics of C3 synthesis

We determined the kinetics of C3 synthesis on day 4 after addition of PMA to the culture medium. The U-937 cells were fed with fresh medium [previously equilibrated for 2 h at 37 °C in a CO_2/air (1:19) atmosphere] and a portion of supernatant was harvested every hour over a 6 h period, and then 24 h and 48 h later without changing the medium. Fig. 2, which represents the cumulative production of C3 in the medium, shows that C3 synthesis regularly increased until the fifth hour.

In another series of experiments, cycloheximide was added at the beginning of the 6 h period. A portion of supernatant was removed at 3 h and 6 h. At the sixth hour, the cells were gently rinsed and cultured in medium without cycloheximide. Supernatants were tested at 24 h and 48 h.

As shown in Table 1, no C3 was detected at 3 and 6 h, indicating that cycloheximide completely blocked C3 synthesis during the incubation period. After removal of the inhibitor, the cells recovered the capacity of producing a significant quantity of C3 (120 ng/ml at 24 h; 140 ng/ml at 48 h).

Haemolytic assay for newly synthesized C3

The functional activity of the newly synthesized C3 protein was investigated by haemolytic assay. The U-937 cells were grown for 4 days in the presence of PMA without changing the medium. On day 5, the cells were fed with fresh medium, and portions of supernatant were harvested over a period of 7 h for e.l.i.s.a. and haemolytic assay. The results of these experiments are presented in Table 2.

The specific activity of fresh normal human serum was determined in parallel and was estimated to be 1.5×10^5

Table 1. Blockade of C3 synthesis by cycloheximide and recovery after 2 days in fresh medium

The U-937 cells were incubated for 6 h with cycloheximide (5 μg/ml), washed twice and resuspended in RPMI/10% (v/v)-FCS medium without cycloheximide. Abbreviation used: NS, not significant (lower than 3 ng/ml).

Time (h)	C3 (ng/10^6 cells)	
	No cycloheximide	+Cycloheximide
0	3	NS
3	55	NS
6	160	NS
24	200	120*
48	190	140*

* Removal of cycloheximide.

Table 2. Haemolytic activity of C3 synthesized by PMA-stimulated U-937 cells

The U-937 cells were grown for 4 days in the presence of PMA without changing the medium. On day 5 the cells were fed with fresh medium and portions of supernatant were harvested over a period of 7 h for e.l.i.s.a. and haemolytic assay.

Time (min)	C3 (ng)*	Specific activity†
30	18	1.1
60	22	1.3
90	26	1.6
120	27	1.5
240	31	1.6
420	50	0.86

* Production of C3 in supernatants of PMA-treated U-937 cells at a concentration of 0.5×10^6/ml, as determined by e.l.i.s.a.

† Expressed as $10^{-5} \times$ effective molecules/ng of C3.

effective molecules/ng of C3. The C3 produced by stimulated U-937 cells exhibited a specific activity similar to that of serum C3 during the first 4 h of culture (Table 2). At 7 h, a 57% ±20% decrease in specific activity was observed.

SDS/polyacrylamide-gel-electrophoretic analysis of C3

At 4 days after addition of PMA, U-937 cells were cultured with [^{35}S]methionine. After 1 h in methionine-free medium, [^{35}S]methionine was added and the supernatants were harvested after 15, 30, 60 and 90 min. Each portion was mixed with affinity-purified anti-C3c (1 mg/ml) and protein A–Sepharose to immunoprecipitate the labelled C3. Immunoprecipitates were analysed by SDS/polyacrylamide-gel electrophoresis (Fig 3). C3 was characterized by two radioactive bands of M_r 110000 and 75000 corresponding to the α- and β-chains respectively. A significant extracellular accumulation of C3 was obtained as early as 30 min and increased until 90 min. When culture supernatants were treated with protein A–Sepharose and an irrelevant antibody, a weak background was seen without well-defined bands.

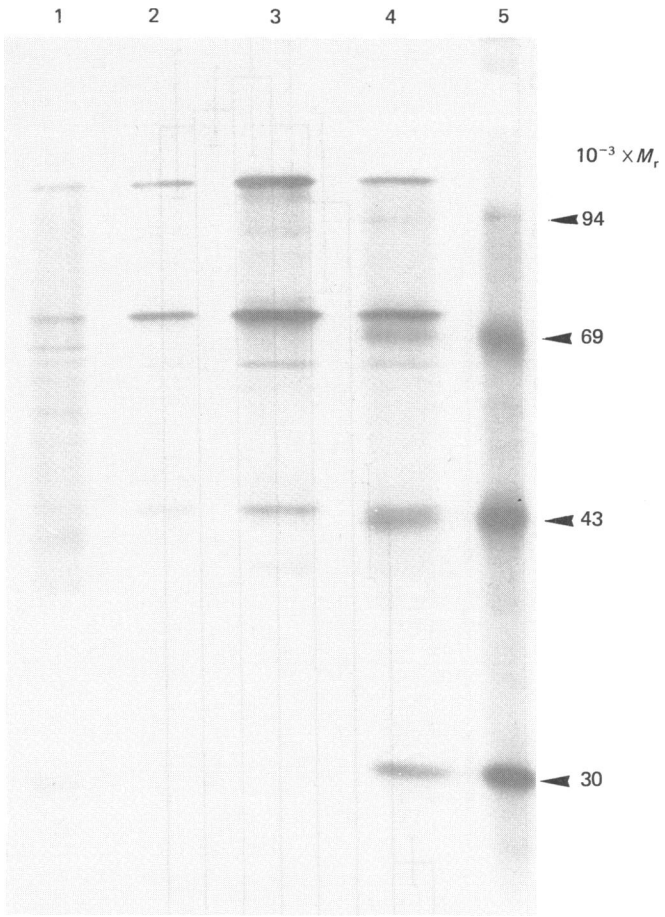

Fig. 3. Autoradiograph of SDS/polyacrylamide-gel electrophoresis of ^{35}S-labelled C3 produced by U-937 cells

After 4 days in the presence of PMA the cells were pulsed for 15–90 min with [^{35}S]methionine, and the supernatants were immunoprecipitated by rabbit anti-(human C3) IgG. The immunoprecipitates were analysed by SDS/polyacrylamide-gel electrophoresis under reducing conditions. Lanes 1–4, supernatants corresponding to 15, 30, 60 and 90 min of pulse; lane 5, markers ($10^{-3} \times M_r$).

Over a long period of [^{35}S]methionine incorporation other radioactive bands appear (Fig. 2, lane 4), of M_r 68000, 43000 and 30000, with a concomitant decrease of the α-chain (M_r 110000) intensity. These bands are characteristic of C3 as well, since inhibition of precipitation with a 100-fold molar excess of unlabelled C3 inhibited the precipitation of these bands (results not shown). These bands are characteristic of iC3b (M_r 68000, 43000, 75000) and C3c (75000, 43000, 27000) fragments.

In order to study the intracellular precursor form of C3, the cells were pulse-labelled for 1 h with [^{35}S]methionine. The medium was then replaced with medium containing 3 mM unlabelled methionine. Supernatants were harvested at subsequent intervals and C3 was immunoprecipitated from either detergent-solubilized cells or from culture media.

As shown in Fig. 4, during the first 30 min period we observed in the intracellular lysates a high-M_r (180000) species, which subsequently disappeared. [In this experiment we chose electrophoresis conditions to reveal

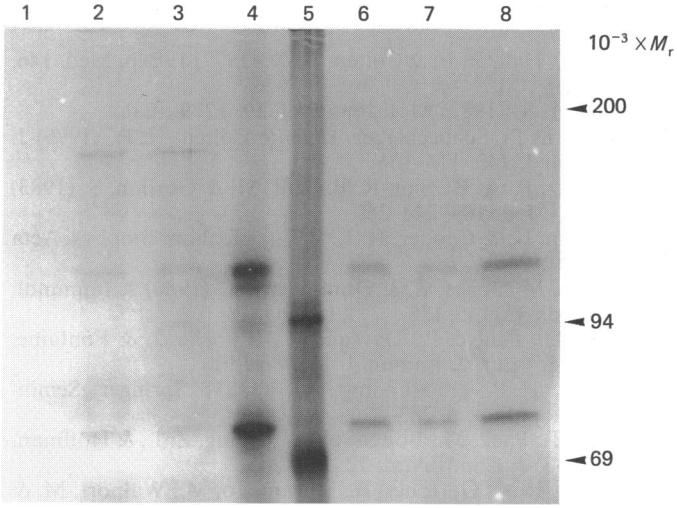

Fig. 4. Autoradiograph of SDS-polyacrylamide gel electrophoresis of biosynthetically labelled C3 immunoprecipitated from U-937 cell lysates and supernatants

U-937 cells were pulsed for 1 h with [^{35}S]methionine and chased with excess unlabelled methionine for 15, 30 and 60 min. Lanes 1–3, lysates corresponding to 60, 30 and 15 min of chase; lanes 6–8, supernatants corresponding to 15, 30 and 60 min of chase; lane 4, ^{125}I-labelled C3; lane 5, markers ($10^{-3} \times M_r$).

high-M_r species (7.5%-acrylamide running gel).] We also noted a progressive appearance of the characteristic bands of C3 (M_r 110000 and 75000) in the extracellular medium after 30 min of chase. These bands could be observed in the lysates too, and may reflect the presence of C3 adsorbed on the membrane of the cells.

DISCUSSION

There is increasing evidence for participation of cells from the monocyte/macrophage lineage in the synthesis of complement components, although information about its physiological role is still scarce (Whaley, 1980; Hartung & Hadding, 1983).

We focused our study on a human continuous cell line, U-937, established in 1976 by Sundstrom & Nilsson (1976), which is considered to be closely related to monocytes. Production of complement components by this cell line has not yet been widely investigated, although the production of C2 component (Littman *et al.*, 1983) and factor D (Barnum & Volanakis, 1985) has been reported.

In the present paper we describe the synthesis of the third component of complement (C3) induced by PMA, a tumour promotor capable of inducing maturational changes in various monocyte-like cell lines, including U-937 (Koren *et al.*, 1979; Hattori *et al.*, 1983).

When PMA was added to the culture medium, U-937 cells underwent remarkable morphological changes, as assessed by inverted-microscope observation of the cultures: the cells strongly adhered to the plastic tissue-culture flasks and became 'macrophage-like' cells within 3 days after addition of PMA. This observation is consistent with previous findings showing changes within 24 h in PMA-treated U-937 cells, as well as the appearance of new antigens on the differentiated cells (Hattori *et al.*, 1983).

Stimulation of U-937 cells by PMA produced a significant and consistent increase in C3 production, as assessed by e.l.i.s.a. and incorporation of [^{35}S]methionine into newly synthesized protein. This synthesis was detected on day 3 after addition of PMA and persisted for about 1 week. A plateau (162 ng of C3/24 h per 10^6 cells) was reached on day 6 or 7, when harvesting the whole medium every 24 h, and evidence for synthesis *de novo* was provided by cycloheximide inhibition and recovery of C3 production after removal of the inhibitor. Kinetic study showed that, after being differentiated into macrophages (up to 4 days of culture in the presence of PMA), the U-937 cells synthesized increasing amounts of C3 only for 6 h. The cumulative C3 production in the medium reached a plateau of 160 ng of C3/10^6 cells in 6 h, and remained stable over 48 h. This synthesis is rather higher than that described for *Corynebacterium parvum*-stimulated guinea-pig peritoneal macrophages (180 ng/12 h per 10^6 cells) (Zimmer *et al.*, 1982), and lower than that recently reported for murine macrophages exposed to lymphokines containing macrophage-activating factor (44 ng/12 h per 10^5 cells) (Koestler *et al.*, 1984). When the culture medium was not changed, the amount of C3 remained stable for 6–48 h. These observations could indicate that C3 exerts a feedback effect on C3 synthesis; such a mechanism was observed for C4 synthesis by guinea-pig peritoneal macrophages (Auerbach *et al.*, 1984). Alternatively, C3 could undergo an extra- or intra-cellular degradation, leading to a constant level of C3 in the extracellular medium. This second hypothesis was strengthened by the results of some of our experiments (see below).

SDS/polyacrylamide-gel-electrophoretic analysis after pulse labelling showed that C3 synthesized by U-937 cells had the same size and subunit structure as the plasma form of C3. This finding is consistent with previous observations in guinea-pig macrophages (Bentley *et al.*, 1978) and human monocytes (Ezekowitz *et al.*, 1983). Pulse–chase experiments showed a band of M_r 180000 in the intracellular lysates, which was assumed to be pro-C3, the precursor form of C3. Such a precursor was described in guinea-pig liver homogenates (Brade *et al.*, 1977) and in a hamster fibroblastic cell line (Senger & Hynes, 1978).

The C3 synthesized by U-937 cells was functional as assessed by a haemolytic assay. Its specific activity (1.5×10^5 effective molecules/ng of C3) was similar to that of plasma C3 during the first 4 h of culture. In contrast, after 7 h of culture, the newly produced C3 had lost about 50% of its activity. Our observations are consistent with the findings of Strunk *et al.* (1983), who showed a loss of haemolytic activity of purified C3 when added to the monocyte cultures, and could be explained either by spontaneous hydrolysis of the thioester bond in the C3 molecule (Pangburn *et al.*, 1977) or by enzymic cleavage of C3 by other products synthesized by the cells.

In our experiments we noted the presence of bands of 68000 and 43000 M_r that are characteristic of iC3b. They appeared as early as 60 min and may indicate cleavage of C3 by U-937 cells. Nevertheless, no loss of haemolytic activity was detected up to 4 h. This discrepancy in time may be related to the incubation period for immunoprecipitation, which exceeds 18 h, and could amplify the degradation process. The hypothesis of cleavage of newly

secreted C3 by U-937 cells themselves is supported by several observations. C3 could be cleaved into C3b by two processes. First, U-937 could secrete all the components of the alternative pathway, as do human monocytes, and assemble a functional C3 convertase (Ezekowitz *et al.*, 1983). Recently, Barnum & Volanakis (1985) found secretion of factor D by U-937 cells. Secretion of factor B by these cells was not reported up until now. Second, C3b can be generated by cleavage of C3 by elastase or other proteinases. U-937 cells synthesize elastase, but failed to secrete it efficiently (Senior *et al.*, 1982). Nevertheless, no data are available concerning the secretion of elastase by PMA-activated U-937 cells.

The processing of C3b into iC3b needs the presence of factor I and of a cofactor (factor H or CR1). There is no evidence that U-937 cells could secrete factor I, but when cultured in the presence of FCS, these cells could adsorb a large amount of calf factor I and release it in media containing no FCS. We showed that U-937 cells cleaved C3b into iC3b in the presence of factor H and FCS, even after three washings with phosphate-buffered saline, but lost this capacity after several washings (Gilbert *et al.*, 1985).

In the present study factor I was probably supplied by FCS adsorbed on to U-937 cells. The cofactor activity was probably expressed on the membrane of U-937 cells. These cells express CR1 (Tedder *et al.*, 1983), but only 5–10% of the cells were labelled with a monoclonal anti-CR1 antibody (Hogg *et al.*, 1984). In our hands, quiescent U-937 cells did not express CR1, but after stimulation by PMA, low amounts of CR1 (< 1000 copies/cell) were detectable (Gilbert *et al.*, 1985). Moreover, a membrane form of factor H on U-937 cells was recently observed by Malhotra & Sim (1985) and by ourselves (D. Gilbert & M. Fontaine, unpublished work). Whatever the sources of the alternative pathway components, in our model, all these components could be present and could account for cleavage of C3 into C3b.

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